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Comparison of WI-38, MRC-5, and IMR-90 Cell Strains for Isolation of Viruses from Clinical Specimens

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With the diminishing supply of the human fetal lung WI-38 cell strain, a replacement for viral isolation is needed. Two candidates are the human fetal lung strains MRC-5 and IMR-90. A comparison of WI-38, MRC-5, and IMR-90 was performed to evaluate efficiency and speed of viral isolation, clarity of cytopathic effect, and ease of growing the cells. The inocula were clinical specimens rather than tissue culture-adapted isolates. Frozen samples of 46 specimens that had previously yielded an isolate on WI-38 were thawed and inoculated onto WI-38, MRC-5, and IMR-90 cells. In addition, 95 freshly taken clinical specimens of undetermined infectivity were inoculated onto the cell strains. Viral recovery rates were similar on all three strains, as were the appearance and speed of onset of the cytopathic effect. MRC-5 and WI-38 cells remained healthy until generation 36, whereas IMR-90 cells went into crisis by generation 20. The longer life span of the MRC-5 cells makes them more suitable than IMR-90 cells to replace the WI-38 strain for routine use in viral diagnosis.

The WI-38 strain of human embryonic lung cells is in short supply (13). This strain has been in wide use for years as one of the standard cell strains for viral isolation work (5) and more recently for production of viral vaccines (2, 11, 12). In diagnostic virology this strain has been relied upon for isolation of viruses belonging to the herpes family (herpes simplex types 1 and 2, cytomegalovirus [CMV], varicella-zoster [VZ] [6]), numerous enteroviruses (4, 7), rhinoviruses (3), and to a lesser extent other respiratory viruses (1). Other fibroblast cell strains are now needed to replace WI-38. Several candidate strains derived from human embryonic lung have been well characterized, including MRC-5, a strain derived from a 14-week-old male fetus (8), and IMR-90, a strain obtained from a 16-week-old female fetus (10). In the present study WI-38, MRC-5, and IMR-90 cells were compared for ability to recover viruses from clinical specimens, for speed of development of cytopathic effect (CPE), for clarity of CPE morphology, and for in vitro longevity.

MATERIALS AND METHODS

Comparison of efficiency of viral isolation on WI-38, MRC-5, and IMR-90 cells by using clinical specimens known to yield an isolate on WI-38 cells. A total of 52 clinical specimens previously yielding an isolate on WI-38 cells were chosen for study. All inocula were samples of original clinical specimens that had been stored at −70°C shortly after collection. When inoculated after thawing, six specimens were negative for virus isolation on all three cell strains and were excluded from the study. The remaining 46 specimens yielded an isolate on at least one cell strain and formed the basis for the comparison.

The cell strains used were WI-38 cells between generations 20 and 34, MRC-5 (MRC-5 cells were supplied by Pat Jacobs of the Medical Research Council, London, England) cells between generations 29 and 36, and IMR-90 (IMR-90 cells were supplied by A. Greene from the Institute for Medical Research, Camden, N. J.) cells between generations 10 and 20. The cells were grown in T 75 plastic flasks containing 25 ml of Eagle minimal essential medium supplemented with 7.5% fetal calf serum, 0.025% bicarbonate, 1% glutamine, 100 U of penicillin per ml, 5 μg of gentamicin per ml, and 0.5 μg of amphotericin B per ml. Cells were passaged by using 0.25% trypsin and 0.1% versene and were split weekly at a 1:4 ratio.

An early passage sample from each cell strain was tested for mycoplasma organisms and found to be free of contamination. WI-38 and MRC-5 cells were tested by the method of Levine (9), whereas IMR-90 cells were analyzed as described by Nichols et al. (10).

For viral isolation experiments, cells were grown in 15-cm screw-capped tubes maintained in 2 ml of Eagle minimal essential medium supplemented with 2% fetal calf serum, 0.025% bicarbonate, 1% glutamine, 150 U of penicillin per ml, 10 μg of gentamicin per ml, and 1.5 μg of amphotericin B per ml. Specimens were inoculated in 0.1-ml volumes onto three tubes of each cell strain. Inocula were placed directly onto the monolayers without allowing for a period of viral adsorption onto medium-free cells. Cultures were kept stationary at 37°C in an atmosphere of 5% CO_2 in air. Tubes were

examined five times weekly for CPE, except for cytomegalovirus (CMV)-inoculated cultures, which were observed twice weekly. Cultures inoculated with herpes simplex types 1 or 2 were kept 7 days, enteroviruses were kept 14 days, and VZ and CMVs were kept 28 days. Cultures were not blind passaged. The day on which CPE first appeared was noted. The clarity of the CPE was compared among the three cell strains.

The sources of specimens were as follows: herpes simplex type 1—six throat swabs and two swabs of lip lesions; herpes simplex type 2—eight swabs of vesicle fluids (genital, skin, finger); CMV—nine urines; VZ—six swabs of vesicle fluids; and enteroviruses (echo 1, 5, 6, 9, 22, Coxsackie A9, A16, polio 1)—seven rectal swabs, three cerebrospinal fluids, four throat swabs, and one urine. All swabs were placed in 2 ml of transport medium (Hanks balanced salt solution with 0.5% gelatin added), which served as the source of the inoculum.

Viruses were identified as follows: herpes simplex types 1 and 2 by chicken embryo fibroblast plaque morphology (14), VZ and CMV by the characteristic cell morphology, and enteroviruses by immune serum neutralizations (performed by the Viral Laboratory, Pennsylvania Department of Health). On reisolation of viruses from the three cell strains, viral identification was based on the morphology of the CPE. By this criterion, the reisolates were all identical to the original viral isolates.

Comparison of efficiency of viral isolation on WI-38, MRC-5, and IMR-90 cells by using fresh clinical specimens of undetermined infectivity. Immediately after collection, 49 specimens were inoculated onto the three cell strains, and an additional 46 specimens were inoculated onto two of the strains, WI-38 and MRC-5. The types of specimens selected were consecutive samples arriving in the laboratory for routine isolation over a 3-week period. These included 28 urine, 21 rectal, 15 respiratory secretion, 12 vesicle fluid, 11 cerebrospinal fluid, 3 autopsy or biopsy tissue, 2 buffy coat, and 3 miscellaneous samples. Cultures were examined for CPE three times weekly for 4 weeks. Isolates were identified as indicated above. Respiratory syncytial virus was identified by the characteristic CPE produced in HEp-2 cells, and by indirect fluorescent-antibody staining (conjugate obtained from Burrough Wellcome Co.).

RESULTS

Comparison of WI-38, MRC-5, and IMR-90 by using inocula previously yielding a virus on WI-38 cells. From the frozen samples of 46 specimens originally yielding an isolate on WI-38 cells, virus was recovered on WI-38, MRC-5, and IMR-90 40, 42, and 42 times, respectively (Table 1). Because each specimen was inoculated in triplicate, a total of 138 culture tubes were inoculated for each cell strain. Table 1 also shows that the number of culture tubes yielding an isolate was similar for all three cell strains.

The rapidity with which CPE developed was compared among the three cell strains (Table 2). In most cases (84 cultures), CPE occurred on the same day on the various cell strains. CPE appeared earlier more often on IMR-90 (21 times) and MRC-5 (20 times) than on WI-38 (13 times); however, these differences were not significant ($P < 0.50$) when evaluated by a nonindependent chi-square test.

Table 3 shows the mean day of onset of CPE

TABLE 1. *Viral isolation on various strains of human embryonic lung cell cultures*

Virus inoculated	No. of specimens/no. of culture tubes inoculated	No. of virus isolations		
		WI-38 (specs/tubes)[a]	MRC-5 (specs/tubes)[a]	IMR-90 (specs/tubes)[a]
Herpes simplex type 1	8/24	8/19	8/21	8/22
Herpes simplex type 2	8/24	7/19	7/19	8/21
CMV	9/27	8/21	8/18	8/17
VZ	6/18	3/8	6/12	3/8
Enteroviruses	15/45	14/40	13/38	15/37
Total	46/138	40/107	42/108	42/105

[a] Number of times a specimen yielded an isolate/number of times a culture tube yielded an isolate (each specimen was inoculated onto three tubes).

TABLE 2. *Speed of detecting CPE on various cell strains*[a]

Comparing cell strains	No. of times CPE noted first on:			Simultaneous onset of CPE
	WI-38	MRC-5	IMR-90	
WI-38 vs MRC-5	5	11		30
WI-38 vs IMR-90	8		13	25
MRC-5 vs IMR-90		9	8	29
Total	13	20	21	84

[a] Nonindependent chi-square test indicates the observed differences are not significant ($P < 0.50$).

TABLE 3. *Mean day of onset of CPE for specimens producing CPE on all three cell strains*

Virus	No. of specimens	Mean day of onset		
		WI-38	MRC-5	IMR-90[a]
Herpes simplex type 1	8	3.9	4.5	2.6
Herpes simplex type 2	7	1.4	1.4	1.4
CMV	7	10.4	8.4	7.7
VZ	2	6.0	5.0	5.0
Enteroviruses	13	3.0	3.4	4.2
Avg	37 (total)	4.5	4.3	4.0

[a] Analysis of variance indicates that the observed differences are not significant.

for the various types of viruses on the three cell strains. By performing an analysis of variance, the differences noted were found to be not significant ($P < 0.15$).

The development of nonspecific (toxic) changes delayed recognition of early CPE more often on WI-38 and IMR-90 cells than on MRC-5 cells. This was especially common for CMV, VZ, and enterovirus CPE which, in the early stages, was most distinct on MRC-5 cells. However, well-developed or late CPE was similar in appearance on all three cell strains.

The growth characteristics and life spans of the MRC-5 and WI-38 cells were similar to one another but different from those of IMR-90 cells. WI-38, MRC-5, and early generations of IMR-90 cells, when split 1:4, reached confluency in T 75 flasks in 4 to 5 days. However, by generation 18 of IMR-90 cells, the flasks were not yet confluent at 7 days, and by generation 20 the cells were granular and had gone into crisis (phase III growth). WI-38 and MRC-5 cells grew well and were useful for viral isolation studies beyond generation 36. The short life span of the IMR-90 cells was noted by using two different lots of cells received in the laboratory 1 year apart. Samples of the second lot of IMR-90 cells were split at a 1:2 ratio and these, too, went into crisis by cell generation 20.

Comparison of WI-38, MRC-5, and IMR-90 cells by using inocula of previously undetermined infectivity. A total of 49 fresh clinical specimens of unknown infectivity were inoculated onto the three cell strains. A total of 45 were negative, and 3 were positive (respiratory syncytial virus from two specimens, CMV from one specimen) on all three cell strains. From one urine sample, CMV was isolated on MRC-5 and IMR-90 cells but not on WI-38 cells (Table 4).

A total of 46 additional specimens were inoculated onto two of the cell strains, WI-38 and MRC-5. A total of 40 specimens were negative, and 6 specimens were positive (CMV from 5 specimens and echo 17 from one specimen) on both cell strains (Table 4).

TABLE 4. *Comparison of WI-38, MRC-5, and IMR-90 cells by using fresh clinical specimens*

Cell strain	Specimens[a] inoculated on all 3 cell strains		Specimens[b] inoculated on WI-38 and MRC-5	
	Positive	Negative	Positive	Negative
WI-38	3	46	6	40
MRC-5	4	45	6	40
IMR-90	4	45		

[a] Total of 49 specimens.
[b] Total of 46 specimens.

DISCUSSION

The focus of this comparison was on clinical specimens rather than on isolates that had already been adapted to tissue culture growth. In the first part of the study, the selection of specimens was biased to favor WI-38 cells in that the inocula were all from specimens known to yield an isolate on WI-38. Despite this bias, the results of viral recovery were similar on the three cell strains. In the second comparison, clinical specimens that had not previously been inoculated onto cell cultures were used. Once again the viral isolation results were similar on the three cell strains (Table 4). It appears then that MRC-5 and IMR-90 cells are neither more, nor less, sensitive than WI-38 cells for viral recovery.

The speed with which viral CPE occurred was similar on the three cell strains (Tables 2 and 3). Morphology of CPE was comparable on the three strains, although early CPE was generally easier to detect on MRC-5 cells.

There was one drawback to the use of IMR-90 cells. The cells went into crisis (phase III growth) by cell generation 20. This occurred in two separate lots of cells grown in the laboratory months apart. This short life span compared poorly with WI-38 and MRC-5 cells that remained in phase II growth and looked "healthy" beyond generation 36. Whether subsequent lots of IMR-90 cells will behave similarly is presently under investigation.

These findings indicate that MRC-5 and IMR-90 cells are as efficient as WI-38 cells in detecting viruses from clinical specimens and that CPE develops as rapidly and is as easy to read on the new cell strains as on WI-38 cells. However, the longer life span of MRC-5 cells makes them more suitable than IMR-90 as a replacement for WI-38 for use in diagnostic virology.

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